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(54) Peptide inhibitors of the p33cdk2 and p34cdc2 cell cycle regulatory kinases and human papillomavirus E7 oncoprotein.

(57)   Novel peptide and peptide mimetic ligands which act as inhibitors of p34^{cdc2} kinase, p33^{cdk2} kinase and human papillomavirus transforming protein E7 (HPV E7) are disclosed. The inhibitors are derived from the binding domains of a retinoblastoma tumor suppressor protein (Rb), p107 and a cyclin.

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Technical Field

The present invention relates generally to peptide inhibitors of cell growth and proliferation. More partic-. ularly, the invention relates to ligands which bind to p34^{cdc2}/p33^{cdk2} kinase and inhibit the activity thereof, and which inhibit the specific interaction between retinoblastoma tumor suppressor protein and human papillomavirus transforming protein E7.

Background of the Invention

Cancer cells are characterized by their ability to proliferate in a continuous, uncontrolled fashion. It is now clear that primary cell cycle regulators must be circumvented or directly involved in oncogenesis in order for this to occur.

Cell cycle regulation occurs at the boundaries of the G$_1$/S and G$_2$/M phases, two major transition points of the cell cycle. A key regulator of these transitions is p34^{cdc2} kinase which is known to phosphorylate a number of proteins including histone H1, DNA polymerase α, RNA polymerase II, retinoblastoma tumor suppressor protein (Rb), p53, nucleolin, cAb1, SV40 large T antigen and lamin A. For example, p34^{cdc2} kinase activity is required for entry of cells into mitosis, i.e., for passage from the G$_2$ phase of the cell cycle into the M phase. (Lee et al. (1988) *Trends Genet.* 4:289-90; Dunphy et al. (1988) *Cell* 54:423-431; Gautier et al. (1988) *Cell* 54:433-439; *see, for review,* Cross et al. (1989) *Ann. Rev. Cell Biol.* 5:341-395; Hunter (1989) *Curr. Opinion Cell Biol.* 1:268-274; Nurse (1990) *Nature* 344:503-508). The activity of p34^{cdc2} kinase is, in turn, regulated by both protein:protein interactions and post-translational modifications. Thus, blockage of either of these mechanisms leads to arrest of the mammalian cell cycle. For example, microinjection of p34^{cdc2} antibodies into serum-stimulated rat fibroblasts causes cells to arrest in G$_2$ and treatment of activated T lymphocytes with p34^{cdc2} antisense oligodeoxynucleotides inhibits DNA synthesis (Furukawa et al. (1990) *Science* 250:805-808; Riabowol et al. (1989) *Cell* 57:393-401). Injection of suc1 protein (a ligand of p34^{cdc2}) into HeLa cells arrests cell growth, presumably by disrupting normal p34^{cdc2} protein:protein interactions (Draetta (1990) *TIBS* 15:378-383). In addition, inhibition of the cdc25 phosphatase with specific antibodies blocks the post-translational modification of p34^{cdc2} and leads to HeLa cell death (Galaktionov et al. (1991) *Cell* 67:1181-1194). Rb, p107 protein and the cyclin (cyc) protein family have been shown to associate with p34^{cdc2} (and its homolog p33^{cdk2}) (Pines et al.1990) *Nature* 346:760-763; Tsai et al. (1991) *Nature* 353:174-177; Giordano et al. (1989) *Cell* 58:981-990) ; the E2F transcription factor; the adenovirus E1A protein; and the human papillomavirus transforming protein E7 (Whyte et al. (1988) *Nature* 334:124-129; Chelappan et al. (1991) *Cell* 65:1053-1061; Bandara et al. (1991) *Nature* 352:249-252; Mundryj et al. (1991) *Cell* 65:1243-1253; Pines et al. (1990), *supra*; Tsai et al. (1991), *supra*; Giordano et al. (1989), *supra*; Shirodkar et al. (1992) *Cell* 68:157-166; Devoto et al. (1992) *Cell* 68:157-166; DeCaprio et al. (1988) *Cell* 54:275-283; Dyson et al. (1989) *Science* 243:934-937; Gage et al. (1990) *J. Virol.* 64:723-730). The binding of cyclins to p34^{cdc2} or p33^{cdk2} is required for kinase activity (Solomon et al. (1990) *Cell* 63:1013-1024; Pines et al. (1990), *supra*; Tsai et al. (1991), *supra*; Giordano et al. (1989), *supra*).

The functional domains of the Rb and p107 proteins have been mapped through both genetic and biochemical means. (Hu et al. (1990) *EMBO J.* 9:1147-1155; Ewen et al. (1991) *Cell* 66:1155-1164; Ewen et al. (1992) Science 255:85-87). An approximately 400 amino acid fragment of Rb and p107, termed the Rb pocket, is responsible for association of these proteins with the DNA tumor virus oncoproteins and cellular ligands. Within this domain are six regions of extensive sequence similarity between Rb and p107. (Ewen et al. (1991), *supra*). Likewise, the cyclins share a large region of sequence similarity spanning approximately 87 amino acids, which has been designated the "cyclin box." (Pines et al. (1989) *Cell* 58:833-846) This domain is thought to play a role in protein:protein interactions and it has been shown that deletion of sequences amino-terminal to this domain do not affect cyclin function. (Murray et al. (1989) *Nature* 339:280-286; Lew et al. (1991) *Cell* 66:1197-1206).

p34^{cdc2} protein:protein interactions are altered in human tumors. For example, the gene encoding the cofactor cyclin A is disrupted in hepatocellular carcinoma (Wang et al. (1990) *Nature* 343:555-557). Also, recent data have demonstrated that cyclin D1 (PRAD1) is within the *bcl-1* locus and is rearranged in parathyroid tumors and some B cell leukemias (de Boer et al. (1993) *Cancer Res.* 53:4148-4152; Motokura et al. (1991) *Nature* 350:512-515). In addition, the *bcl-1* locus is frequently amplified in breast carcinoma and cyclin D1 is overexpressed in mouse skin carcinoma (Lammie et al. (1991) *Oncogene* 6:439-444; Bianchi t al. (1993) *Oncogene* 8:1127-1133; Buckley et al. (1993) *Oncogene* 8:2127-2133). Furthermore, the subunits of the cdk kinases are rearranged in transformed cells when compared to their normal counterpart (Xiong et al. (1993) *Genes and Development* 7:1572-1583). This is the result of the loss or underexpression of waf1/cip1 protein, which is normally a repressor of cdk kinase activities, and is regulated by the p53 tumor suppressor protein (Xiong et al. (1993) *Nature* 366:701-704; Serrano et al. (1993) *Nature* 366:704-707; Gu et al. (1993) *Nature* 366:707-710;

Harper et al. (1993) *Cell* 75:805-816; El-Deiry et al. (1993) *Cell* 75:817-825). These data clearly implicate the alteration of p34^{cdc2} kinase activity in oncogenesis.

Accordingly, inhibitors of p34^{cdc2} activity would be useful in the regulation and control of the continuous, proliferative growth of cancerous cells.

Summary of the Invention

The present invention provides for ligands that inhibit the course of cellular proliferation by preventing progression through the G$_1$/S or G$_2$/M boundaries by inhibition of p34^{cdc2} kinase activity. The ligands are useful as modulators of p34^{cdc2} kinase-mediated cell growth and proliferation and, thus, as agents for studying the mechanisms by which cancer cells proliferate in a continuous fashion and as agents for ameliorating tumors associated with disruption of genes which code for essential cyclins, for example, hepatocellular carcinomas, parathyroid tumors, various B cell leukemias and breast carcinomas or tumors which contain a p53 mutation or loss of waf1/cip1.

Accordingly, in one embodiment, the invention is directed to an inhibitor of p34^{cdc2} cell cycle regulatory kinase activity, or an inhibitor of the kinase activity of a homolog thereof, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin.

In preferred embodiments, the inhibitor is a peptide including the amino acid sequence LCAFYIMAK [SEQ ID NO:_____], a peptide including the amino acid sequence MCSMYGICK [SEQ ID NO:____ _____], a peptide including the amino acid sequence CAFYI [SEQ ID No:_____], or substitutions of these sequences which retain inhibitory activity.

In a further embodiment, the invention is directed to an inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin.

In another preferred embodiment, the invention is directed to a complex comprising:

(a) p34^{cdc2} or a homolog thereof; and

(b) an inhibitor of p34^{cdc2} cell cycle regulatory kinase activity, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin, and wherein the complex substantially lacks kinase activity.

In another embodiment, the invention is directed to a complex comprising:

(a) human papillomavirus E7 protein; and

(b) an inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin.

In a further embodiment, the invention is directed to a method of inhibiting p34^{cdc2} cell cycle regulatory kinase activity, or the kinase activity of a homolog thereof, comprising providing a p34^{cdc2} inhibitor as described above and contacting p34^{cdc2} or the homolog thereof with an inhibiting amount thereof.

In an additional embodiment, the invention is directed to a method of inhibiting human papillomavirus E7 activity, comprising providing a human papillomavirus E7 inhibitor and contacting human papillomavirus E7 with an inhibiting amount thereof.

These and other embodiments of the subject invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Figures

Figure 1 depicts a comparison of the amino acid sequences of various p34^{cdc2} binding proteins including cyclins A, B1, C, D and E, p107 and Rb [SEQ ID NOS:_____]. Gaps were introduced to maximize homology, and are represented by dashes. Amino acids are represented by their single letter code. Boxes indicate identical amino acids or conservative changes. The stipled cylinder represents the α-helix II region.

Figure 2 depicts a plot of the percent inhibition of p34^{cdc2} histone H1 kinase activity versus the p107 9mer peptide concentration.

Figure 3 depicts plots of the binding of the p107 30mer peptide to HPV18-E7 (Figure 3A: MBP-E7 (●), MBP alone (Δ)) or competition for the E7-p107 30mer peptide interaction with the p107 9mer peptide (LCA-FYIMAK [SEQ ID NO:_____]) (Figure 3B).

Figure 4 depicts plots of the effect of p107 9mer peptide (LCAFYIMAK [SEQ ID NO:_____]) on the binding of MBP-E7 protein to Rb protein as determined by ELISA (Figur 4A: MBP-E7 (○); MBP alone (Δ)) or by binding competition (Figure 4B: Rb bound ( )).

Detailed Description

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of protein chemistry and biochemistry, molecular biology, microbiology and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature. *See, e.g.,* Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual,* Second Edition (1989); *DNA Cloning,* Vols. I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.K. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL press, 1986); Perbal, B., *A Practical Guide to Molecular Cloning* (1984); the series, *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.).

All patents, patent applications and publications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

A. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "p34^{cdc2}," "p34^{cdc2} kinase" or "p34^{cdc2} cell cycle regulatory kinase", which terms are used interchangeably, is meant an approximately 32-34 kda protein, alternatively known as MPF (maturation/M Phase promoting factor or mitosis-promoting factor), which possesses protein-serine/threonine kinase activity (as described in Lee et al. (1987), *supra*; Dunphy et al. (1988), *supra*; and Gautier et al. (1988), *supra*). The term encompasses the product of the *cdc2* gene of *Schizosaccharomyces pombe* and the product of the *CDC28* gene from *Saccharomyces cerevisiae*, and homologues found in other species (*see,* Arion et al. (1988) *Cell* 55:371-378; Dunphy et al. (1988), *supra*; Gautier et al. (1988), *supra*; and Labbé et al. (1988) *Cell* 57:253-263), including the human homolog of these proteins, p34^{cdc2} (Lee et al. (1987), *supra*). p34^{cdc2} kinase activity is dependent on association with specific cyclins and post-translational modification of Thr-14 and Tyr-15 amino acid residues.

The term "p33^{cdk2}," "p33^{cdk2} kinase" or "p33^{cdk2} cell cycle regulatory kinase" refers to a cyclin-dependent kinase homolog of p34^{cdc2} kinase, the activity of which is similarly dependent on association with one or more cyclin molecules (Tsai et al. (1991), *supra*; Rosenblatt et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:2824-2828; Elleage et al. *Proc. Natl. Acad. Sci. USA* 89:2907-2911).

By "a p34^{cdc2} binding domain" is meant that portion of the molecule in question (i.e., an Rb, p107 or a cyclin) which interacts either directly or indirectly with p34^{cdc2} kinase thereby preventing the activation of the kinase. The binding domain may be a sequential portion of the molecule, i.e., a contiguous sequence of amino acids, or it may be conformational, i.e., a combination of non-contiguous sequences of amino acids which, when the molecule is in its native state, forms a structure which interacts with p34^{cdc2} kinase.

By being "derived from" a binding domain is meant any molecular entity which is identical, substantially homologous, complementary or otherwise functionally or structurally equivalent to the native p34^{cdc2} binding domain of the molecule in question (i.e., an Rb, p107 or a cyclin). Thus, a molecule derived from a particular binding domain may encompass the amino acid sequence of a naturally occurring ligand-binding site, any portion of that binding site, or other molecular entity that functions to bind to an associated ligand. A molecule derived from such a binding domain will interact either directly or indirectly with p34^{cdc2} kinase in such a way as to mimic the native binding domain. Such molecular entities may include competitive inhibitors, peptide mimetics and the like.

An "inhibitor of p34^{cdc2} kinase activity" or an "inhibitor of p33^{cdk2} kinase activity" is intended to mean a peptide or peptide fragment derived from a p34^{cdc2} binding domain of an Rb, p107 or a cyclin, which interacts either directly or indirectly with p34^{cdc2} or p33^{cdk2} and prevents activation of the kinase. If the inhibitor is a cyclin protein, it will not include the full-length sequence of the wild-type molecule. An inhibitor can prevent cyclin-dependent kinase activation by competitively inhibiting the binding of cyclins to p34^{cdc2}. In addition, peptide mimetics, synthetic molecules with physical structures designed to mimic structural features a particular peptide, may similarly serve as inhibitors of kinase activity. Such inhibitors diminish the p34^{cdc2} or p33^{cdk2} enzymatic catalysis of the transfer of the terminal phosphate of ATP, or a similar ATP analog or other nucleotide triphosphate, to a suitable substrate. Such substrates include histone H1, DNA polymerase α, RNA polymeras II, Rb, p53, nucleolin, cAb1, SV40 large T antigen, lamin A and the like. Alternatively, such inhibitors may diminish the ability of activated (e.g., dephosporylated) p34^{cdc2} to support progression through the cell cycle as measured by a suitable cell cycle assay (*see, e.g.,* Pines et al. (1989), *supra*). Such inhibition may be by a direct, competitive mechanism, or by an indirect, non- or uncompetitive mechanism.

An "inhibitor of human papillomavirus E7 protein" is a molecule which interacts with transforming protein E7, either directly or indirectly, to prevent the formation of the specific complex with Rb. As with inhibitors of p34cdc2 kinase, these inhibitors can prevent binding by competitive inhibition, or by an indirect, non-or uncompetitive mechanism. In addition, the term encompasses peptide inhibitors as well as peptide mimetics.

The inhibitory activity of a candidate peptide or peptide mimetic can be tested by assessing the ability of the candidate to bind to the target acceptor, i.e., p34cdc2, p33cdk2 or HPV E7. For example, those candidates which bind to the target acceptor with suitable affinity and specificity can be assayed for their ability to inhibit the activity of the target acceptor. For p34cdc2 and p33cdk2, the ability of the inhibitor to diminish kinase activity can be assessed. For HPV E7, the ability of a candidate inhibitor to compete with Rb for binding to E7 can be determined. Finally, to assess the inhibitory activity in a whole cell setting, the ability of a candidate inhibitor to prevent the progression of a synchronized population of transformed cells through the cell cycle can be determined. For example, HeLa cells can be synchronized using techniques well-known in the art (see, Lew et al. (1991), supra). The inhibition of cell cycle progression through the G_1/S boundary is assessed by a diminution of DNA synthesis in the cell population. Inhibition of cell cycle progression through the G_2/M boundary is determined by the absence of mitotic activity (i.e., cell division).

"Peptide mimetics" are structures which serve as substitutes for peptides in interactions with acceptor molecules (see Morgan et al. (1989) Ann. Reports Med. Chem. 24:243-252 for a review of peptide mimetics). Peptide mimetics, as used herein, include synthetic structures which may or may not contain amino acids and/or peptide bonds, but retain the structural and functional features of a peptide ligand. The term, "peptide mimetics" also includes peptoids and oligopeptoids, which are peptides or oligomers of N-substituted amino acids (Simon et al. (1972) Proc. Natl. Acad. Sci. USA 89:9367-9371). Further included as peptide mimetics are peptide libraries, which are collections of peptides designed to be of a given amino acid length and representing all conceivable sequences of amino acids corresponding thereto. Methods for the production of peptide mimetics are described more fully below.

Two polypeptide sequences are "substantially homologous" when at least about 85% (preferably at least about 85% to 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule. As used herein, substantially homologous also refers to sequences showing identity to the specified polypeptide sequence.

The terms "polypeptide", "peptide" and "protein" are used interchangeably and refer to any polymer of amino acids (dipeptide or greater) linked through peptide bonds. Thus, the terms "polypeptide", "peptide" and "protein" include oligopeptides, protein fragments, analogues, muteins, fusion proteins and the like.

The following single-letter amino acid abbreviations are used throughout the text:

Alanine	A	Arginine	R
Asparagine	N	Aspartic acid	D
Cysteine	C	Glutamine	Q
Glutamic acid	E	Glycine	G
Histidine	H	Isoleucine	I
Leucine	L	Lysine	K
Methionine	M	Phenylalanine	F
Proline	P	Serine	S
Threonine	T	Tryptophan	W
Tyrosine	Y	Valine	V

B. General Methods

Central to the present invention is the discovery of peptide molecules which bind to and inhibit p34cdc2 kinase activity. These molecules are derived from the binding domain of an Rb, p107 or a cyclin. These proteins, depicted in Figure 1, bind p34cdc2, its homolog p33cdk2, the E2F transcription factor and the adenovirus E1A transforming protein. In addition, Rb forms a specific complex with the transforming protein of papillomavirus E7. The peptide inhibitors, and peptide mimetics thereof, provide useful tools for the analysis of the normal

function of p34^{cdc2} and p33^{cdk2} kinases and transforming factors, such as E7, in cell growth and proliferation in precancerous and cancerous cells. Additionally, since the activity of p34^{cdc2} kinase is essential for the progression through the G_1/S and G_2/M transition phases of the cell cycle, the protein inhibitors, or mimetics thereof, can be administered to cancerous tissues in order to suppress tumor growth.

As shown in the examples, the claimed inhibitors of p34^{cdc2} kinase, p33^{cdk2} kinase or HPV E7 include sequences of amino acids derived from the binding domains of proteins which interact with p34^{cdc2} and/or HPV E7, and mimetics thereof. In particular, the inhibitors are derived from a region of homology found in a number of p34^{cdc2} binding proteins known as the α-helix II region, shown in Figure 1. A 30 amino acid peptide, FEFTLVHCPDLMKDLMKDRHLDWLLLCAFYIMAK, [SEQ ID NO:_____] encompassing the α-helix II sequence from p107 has been shown herein to bind to p34^{cdc2} and p33^{cdk2}. This 30 amino acid peptide also inhibits the histone H1 kinase activity of p34^{cdc2} and p33^{cdk2}. By sequentially deleting amino- or carboxy-terminal amino acids, a five amino acid sequence (CAFYI [SEQ ID NO:_____]) was identified as a minimum sequence which retains inhibitory activity. Furthermore, a peptide having nine amino acids (LCAFYIMAK [SEQ ID NO:_____]), including the 5 amino acid peptide, was shown to bind to p34^{cdc2} with an affinity of approximately 10 μM. Thus, useful p34^{cdc2} inhibitors of the present invention will be derived from at least this 5mer, and can include as many as 50 to 200 or more amino acids, so long as a proper binding conformation is retained.

The above-described 30mer peptide also binds to human papillomavirus (HPV) 18-E7 protein. Furthermore, the 30mer peptide inhibits E7 binding to Rb, as does a nine amino acid peptide derived from Rb (MCSMY-GICK [SEQ ID NO:_____]).

As explained above, all of these peptides, as well as molecules substantially homologous, complementary or otherwise functionally or structurally equivalent to these peptides, may be used for purposes of the present invention.

The peptide inhibitors of the present invention may be synthesized by conventional techniques known in the art, for example, by chemical synthesis such as solid phase peptide synthesis. Such methods are known to those skilled in the art. In general, these methods employ either solid or solution phase synthesis methods, well known in the art. See, e.g., J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology, supra*, Vol. 1, for classical solution synthesis.

As explained above, peptide mimetics which structurally and functionally mimic the peptide inhibitors described above will also find use herein and may be generated using the following strategies and procedures. Generally, mimetics are designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chain moieties by a methyl group or pseudoisosteric groups with different electronic properties (see Hruby et al. (1990) *Biochem. J.* 268:249-262), and by systematic replacement of peptide bonds in the above described peptide inhibitors with amide bond replacements. For example, analogues containing amide bond surrogates may be used to investigate aspects of peptide structure and function, such as rotational freedom in the backbone, intra- and intermolecular hydrogen-bond patterns, modifications of local and total polarity and hydrophobicity, and oral bioavailability.

Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic inhibitor of p34^{cdc2}, p33^{cdk2}, HPV E7 or other acceptor. For example, β,β-disubstituted amino acids may be used to examine the effects of conformational constraints on peptide activity (see, e.g., Manning et al. (1982) *J. Med. Chem.* 25:408-414; Mosberg et al. (1983) *Proc. Natl. Acad. Sci. USA* 106:506-512; Pelton et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:236-239).

The mimetics can include isosteric amide bonds such as Ψ[CH$_2$S], Ψ[CH$_2$NH], Ψ[CSNH$_2$], Ψ[NHCO], Ψ-[COCH$_2$] and Ψ[(E) or (Z) CH=CH] (see, for review, Spatola (1983) in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Volume VII, (Weinstein, ed.), Marcel Dekker, New York, 267-357). Structures which mimic the tetrahedral transition state associated with hydrolysis of a substrate bond can also be present and include hydroxymethylene, fluoroketone moieties and phosphoramidate transition state mimics (Bühlmayer et al. (1988) *J. Med. Chem.* 31:1839; Sham et al. (1988) *FEBS Lett.* 220:299; Matthews (1988) *Acc. Chem. Res* 21:333). The synthetic molecules can also include D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule from enzymatic degradation (see, e.g., Freidinger et al. (1985) in "Peptides: Structure and Function," (Deber et al., eds.), Pierce Chem. Co., Rockford, IL, 549-552; Sawyer et al. (1980) *Proc. Natl. Acad. Sci. USA* 77:5754-5758; Torchiana et al. (1978) *Arch. Int. Pharmacol. Ther.* 235:170-176). Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states, e.g., αα'-and ββ'-substituted cyclic amino acids such as I-aminocyclopentanecarboxylic

acid (cycloleucine) and β,β-cyclopentamethylene-β-mercaptopropionic acid (*see* Hruby et al. (1990), *supra*).

The mimetics can also include mimics of inhibitor peptide secondary structure -- structures which can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins -- including β-turn mimetics, such as phenoxathin ring system, and β-sheet mimics, such as epindolidione structures. Design, synthesis and conformational analysis of an α-helix inducing template has been described (Kemp et al. (1988) *Tetrahedron Lett.* 29:4931; Kemp et al. (1988) *Tetrahedron Lett.* 29:4935).

Similarly, peptoids will find use herein. Peptoids are oligomers of N-substituted amino acids (Simon et al. (1972), *supra*), and can be used as motifs for the generation of chemically diverse libraries of novel molecules, which can then be tested for binding and inhibitory activity against p34^{cdc2}, p34^{cdk2}, HPV E7 or other acceptor molecules. The monomers may incorporate t-butyl-based side-chain and 9-fluorenylmethoxy-carbonyl α-amine protection. Oligomerization of the peptoid monomers may be performed by, for example, *in situ* activation by either benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorphosphate or bromotris(pyrrolidino)phosphonium hexafluorophosphate. Other steps are identical to conventional peptide synthesis using α-(9-fluorenylmethoxycarbonyl)amino acids. Oligopeptoids may be identified which have affinities comparable to the corresponding inhibitory peptides and, thus, are useful in p34^{cdc2} and p33^{cdk2} kinase or HPV E7 binding assays (*see* Simon et al. (1992), *supra*).

Peptide ligands that interact with p34^{cdc2}, p33^{cdk2}, HPV E7 or other protein acceptors can be developed by using a biological expression system (*see* Christian et al. (1992) *J. Mol. Biol.* 227:711-8; Devlin et al. (1990) *Science* 249:404-406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382). The use of such systems allows the production of large libraries of random peptide sequences and the screening of these libraries for peptide sequences that bind to particular proteins. The libraries may be produced by cloning synthetic DNA that encodes random peptide sequences into *Escherichia coli* expression vectors. In the filamentous phage system, foreign peptide sequences can be expressed on the surface of the infectious phage (*see* Smith (1985) *Science* 228:1315-1317; Parmley et al. (1988) *Gene* 73:305-318).

For example, a library may be made by ligating into an appropriate phage, a synthetic DNA fragment containing a degenerate coding sequence(NNK)$_n$, where N stands for an equal mixture of the deoxynucleotides G, A, T, and C, K stands for an equimolar mixture of G and T, and n stands for the number of amino acid residues desired in the product peptide. Affinity purification of phage displaying affector-binding peptides may be done by biotinylating the affector, incubating the phage with the biotinylated affector and reacting the phage on streptavidin-coated plates. Bound phage are eluted and amplified on agar medium and subjected to further rounds of affinity purification. Phage from later rounds of affinity purification are cloned and propagated, their DNAs sequenced to determine the amino acid sequences of their expressed peptide and their binding to p34^{cdc2}, p33^{cdk2}, HPV E7 or other affector molecules assessed by enzyme-linked immunosorbent assays (ELISA). Such libraries consisting of large numbers of clones expressing different short peptide sequences can be used to map binding domains.

Large libraries of peptide inhibitors can also be constructed by concurrent synthesis of overlapping peptides as described in U.S. Patent No. 4,708,871 to Geysen. The synthetic peptides can be tested for interaction with acceptor molecules by ELISA while still attached to the support used for synthesis. The solid support is generally a polyethylene or polypropylene rod onto which is graft polymerized a vinyl monomer containing at least one functional group to produce polymeric chains on the carrier. The functional groups are reacted to provide primary or secondary amine groups which are sequentially reacted with amino acid residues in the appropriate order to build the desired synthetic peptide using conventional methods of solid phase peptide chemistry.

Once produced, the inhibitory peptides or peptide mimetics can be used in pharmaceutical compositions to ameliorate tumors associated with disruption of genes which code for essential cyclins, for example, hepatocellular carcinomas, parathyroid tumors, some B cell leukemias and certain breast carcinomas or tumors associated with the loss of p53 function. The inhibitory peptides of the present invention can be formulated into therapeutic compositions in a variety of dosage forms such as, but not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the particular cancer type targeted. The compositions also preferably include pharmaceutically acceptable vehicles, carriers or adjuvants, well known in the art, such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolyt s such as protamine sulfate. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. Actual methods of preparing such compositions are known, or will be apparent, to those skilled in the art. *See, e.g., Remington's Pharmaceutical Sciences,* Mack Publishing Company, Easton, Pennsylvania, 18th edition, 1990.

The above compositions can be administered using conventional modes of delivery including, but not lim-

ited to, intravenous, intraperitoneal, oral, intralymphatic, or subcutaneous administration. Local administration, to the tumor in question, will also find use with the present invention.

Therapeutically effective doses will be easily determined by one of skill in the art and will depend on the severity and course of the disease, the patient's health and response to treatment, and the judgment of the
5   treating physician.

C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are
10   offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

15   ## Experimental Methods

HeLa Cell Lysates:

HeLa cell extracts served source of p34[cdc2] and p33[cdk2] and were prepared as follows. HeLa cells were
20   lysed in 20 mM N-[2-hydroxyethyl]piperazine-N'-[2-hydroxypropanesulfonic acid] (HEPES), pH 7.0, 150 mM NaCl, 5 mM sodium vanadate, 10 mM $Na_4P_2O_7$, 1 mM $ZnCl_2$, 2 mM ethylene-diaminetetraacetic acid (EDTA), 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 0.25% (v:v) NP-40, 1 mM Benzamidine, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 20 μg/ml each: $N_2S_2O_3$, leupeptin, Antipan and Pepstatin, followed by centrifugation to remove cell debris.

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Immunoprecipitation of p34[cdc2]:

Immunoprecipitation of p34[cdc2] from HeLa cell extracts was performed using previously reported proce-
dures (Harlow et al. (1988) in: *Antibodies: A Laboratory Manual*, ed. Harlow and Lane, Cold Spring Harbor:
30   Cold Spring Harbor Laboratory, pp 421-470) and anti-p34[cdc2] antibody, G6 (Gibco, BRL). Immunoprecipitation by this method yields an activated kinase complex (Draetta et al. (1988), *Cell* 54:17-26).

Assay of p34[cdc2] Kinase Activity:

35   Histone H1 kinase reactions were performed in 50 mM Tris, pH 8.0, 10 mM $MgCl_2$, 0.5 mM DTT, 1 mM EGTA plus 0.1 mM ATP, 1 μCi γ-[^{32}P]-ATP and 10 μg histone H1 (Boeringer Mannheim), and a source of p34[cdc2]. Reactions were incubated at 30°C for 20 minutes and stopped by the addition of protein gel sample buffer. Kinase reactions were separated by SDS-PAGE (12.5% gel). The bands were detected by autoradiography and the amount of ^{32}P incorporated was quantitated using an Ambis Radioanalytic Imaging System.

40

Example 1

Comparison of p34[cdc2] Binding Proteins

45   Inhibitors of p34[cdc2] cell cycle regulatory kinase activity were identified by first comparing the primary amino acid sequence of known p34[cdc2]-associated proteins: human cyclins A, B1, C, D, E, p107 protein and retinoblastoma tumor suppressor protein (Rb) [SEQ ID NOS:_____]. The basic alignment of the human cyclin proteins was demonstrated by Lew et al. (1991), *supra*, and the alignment of Rb and p107 was performed by Ewen et al. (1991), *supra*. The sequences of all seven proteins was subsequently compared, as
50   depicted in Figure 1. Secondary structure analysis of all seven sequences was performed using both Chou-Fasman (Chou et al. (1974) *Biochemistry* 13:211-222; Chou et al. (1974) *Biochemistry* 13:222-245) and Robson-Garnier (Garnier et al. (1978) *J. Mol. Biol.* 120:97-120) structure prediction programs. Two regions of common α-helical structure, designat d α-helix I and α-helix II, were predicted for six of seven proteins by both structure prediction programs.
55   A 170 amino acid region of sequence similarity was observed among Rb, p107 and the human cyclins. Rb and p107 share the highest degree of similarity with cyclin E, 27% and 34%, respectively. These s quences contain two regions of common predicted α-helical structure. Projection of th   α-helix II sequences of Rb, p107 and cyclin E on a helical wheel demonstrates that the conserved residues ar   displayed on one face of the α-

helix.

Example 2

5   Binding of p34cdc2 to p107.

In order to test whether the α-helix II domain is involved in the interaction of Rb, p107 and cyclin E with p34cdc2, a 30 amino acid peptide from p107 (residues 692-721: FEFTLVHCPDLMKDLMKDRHLDWLLLCAFYI-MAK [SEQ ID NO:_____]) encompassing α-helix II, was synthesized and assayed for its ability
10   to associate with p34cdc2. The 30mer was covalently linked to CH-activated sepharose 4B (Pharmacia) as follows. The p107 30 amino acid peptide, which was insoluble in aqueous solutions, was linked to the sepharose in N,N-dimethylformamide. The concentration of 30mer linked to the bead was 0.85 μmole/ml as determined by HPLC. The p107 30mer-sepharose was incubated with total HeLa cell lysate and washed with 25 volumes of lysis buffer. The bound proteins were eluted with increasing concentrations of NaCl, separated by sodium
15   dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12.5% gel) and transferred to polyvinylidene difluoride (PVDF) membranes. p34cdc2 was detected by probing the membrane with anti-p34cdc2 antibody, G6 (Gibco, BRL) (see, Draetta et al. (1988), supra).

The p107 30mer-sepharose binds to p34cdc2 and the majority of the complex was stable to 2M NaCl. These results indicate that the amino acid sequence within α-helix II contains sufficient information to direct interac-
20   tion with p34cdc2.

Example 3

Binding of p33cdk2 to p107

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The ability of p107 to bind to p33cdk2 was examined using the methods described in Example 2. p107 30mer-sepharose beads were incubated with HeLa cell extract (3 x 10^5 cells per reaction) and then washed extensively. Bound complexes were then separated on a 12.5% SDS-PAGE gel and blotted onto a PVDF membrane. The blot was then probed with the kinase antibody. The results of this western blot analysis with anti-
30   p33cdk2 antibody (Upstate Biotechnology) indicates that the p107 30 amino acid peptide binds this kinase subunit.

Example 4

35   Histone H1 Kinase Activity of Bound and Immunoprecipitated p34cdc2.

In order to examine the effect of binding of p34cdc2 to p107 30mer-sepharose on p34cdc2 kinase activity, histone H1 kinase activities associated with p107 30mer-sepharose and immunoprecipitated p34cdc2 were compared as follows. p34cdc2 was bound to p107 30mer-sepharose as described in Example 2. Histone H1 kinase
40   reactions were performed using either immunoprecipitated or p107 30mer-sepharose bound p34cdc2. The amount of p34cdc2 bound to p107 30mer-sepharose was compared with that contained within the immune complex by western blot. Two times the amount of immunoprecipitated p34cdc2 used in the kinase reaction was blotted to facilitate easier detection. The amount of p34cdc2 associated with the p107 30mer-sepharose was determined by densitometry to be ≧ 50 time greater than contained within the immune complexes. This data
45   was used in normalizing the kinase activity associated with the different beads.

The observed histone H1 kinase activity for the p107 30 amino acid peptide-bound enzyme is similar to that of the immune complex. Normalization for the amount of p34cdc2 protein present in both reactions demonstrated that the peptide bound enzyme has a specific activity equal to only 1.4% the specific activity of the immune complex. This is consistent with p107 30mer competing for cyclin binding to p34cdc2, and is indicative
50   of a common mechanism of binding for the cyclins, Rb, and p107.

Example 5

Peptide Inhibition of p34cdc2

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Histone H1 Kinase Activity

As a further test of the association of p107 with p34cdc2, and to define the minimal sequence necessary

for interaction with p34^{cdc2}, the effect of p107 peptides on the p34^{cdc2} immune complex histone H1 kinase activity was assessed. Both amino- and carboxy-terminal deletions were introduced into the original 30 amino acid peptide (*see* Table 1). The peptides were synthesized by standard methods and purified by HPLC. Immune complexes were formed as described in Example 4 and resuspended in kinase buffer plus 50 µM of the re-
5   spective peptide solubilized in N,N-dimethylformamide, or an equal volume of solvent. After incubating the reactions for 15 minutes on ice, histone H1 and γ-[^{32}P]-ATP were added and the kinase assays were performed and quantitated as described in Example 4.

Addition of the p107 peptides inhibits histone H1 kinase activity associated with the immune complex (*see* Table 1). Addition of the p107 peptides to a p33^{cdk2} kinase reaction also inhibits the activity of the kinase.
10   Peptides with deletions of the amino-terminal sequences to Cys$_{713}$ and carboxy-terminal sequences to Ile$_{717}$ retain the ability to inhibit p34^{cdc2} activity. Further deletions to either Ala$_{714}$ or Tyr$_{716}$ resulted in inactive peptides. An unrelated 19mer control peptide had no effect upon histone H1 activity.

These data demonstrate that the minimal sequence which retained inhibitory activity was a pentapeptide with the sequence CAFYI [SEQ ID NO:_____]. Non-conservative amino acid substitutions
15   within the pentapeptide totally abrogated p34^{cdc2} kinase inhibition except at Cys$_{713}$.

Example 6

Effect of Single Amino Acid Substitution on p34^{cdc2} Inhibitory Activity of p107 Peptides
20

In order to examine the role for Cys$_{706}$ of Rb and Cys$_{713}$ of p107 in the binding of p34^{cdc2} and other ligands such as E1A or E2F, a peptide was synthesized which contained the Cys to Phe amino acid substitution at position 713 and its inhibitory activity was examined. This single mutation resulted in a 30% decrease in the peptide's ability to inhibit histone H1 kinase activity, confirming the importance of Cys$_{713}$ in the p107 peptide-
25   p34^{cdc2} interaction (*see* Table 1, 9mer versus 713CF. This residue is also conserved in cyclins D and E and may function in a similar manner. In addition, the substitution of Ala for Tyr$_{716}$, which is highly conserved between the other proteins examined, totally abrogated p34^{cdc2} kinase inhibition (*see* Table 1, 9mer versus 716YA. These data also suggest that the p107 peptides are capable of competing for and displacing cellular factors bound to p34^{cdc2}, which are required for kinase activation (Pines et al. (1989), *supra*; Murray et al. (1989), *supra*;
30   Solomon et al. (1990), *supra*; Draetta et al. (1989) *Cell* 56:829-838) and are consistent with the earlier observation that low levels of kinase activity are associated with the p107 30mer peptide-sepharose.

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TABLE 1				
		[SEQ ID NO.]	p34cdc2 Inhib. (%)	IC$_{50}$ (µM)
30mer	CAFYIMAK	---	ND	ND
21mer	DLMKDRHLDQLLLCAFYIMAK	---	70	ND
18mer	KDRHLDQLLLCAFYIMAK	---	80	ND
14mer	LDQLLLCAFYIMAK	---	80	ND
9mer	LCAFYIMAK	---	90	9
N7mer	AFYIMAK	---	0	ND
N5mer	YIMAK	---	0	>100
C7mer	LCAFYIM	---	70	ND
C6mer	LCAFYI	---	79	ND
C5mer	LCAFY	---	0	nd
NC5mer	CAFYI	---	82	13
NC4mer	CAFY	---	0	ND
713CF	LFAFYIMAK	---	60	15
714AE	LCEFYIMAK	---	0	23
715FA	LCAAYIMAK	---	0	>100
715FK	LCAKYIMAK	---	0	>100
715FY	LCAYYIMAK	---	20	45
716YA	LCAFAIMAK	---	0	40
716YF	LCAFFIMAK	---	80	25
717IK	LCAFYKMAK	---	0	>100
719AQ	LCAFYIMQK	---	75	ND
SV-NC5mer	PKKKRKVCAFYI	---	0	ND
RR-NC5mer	RRCAFYI	---	0	ND
PKG-NC5mer	RKRCAFYI	---	0	ND
SPKK-NC5mer	SPKKGCAFYI	---	0	ND
NC5mer-SPKK	CAFYIGSPKK	---	80	ND

Example 7

Affinity of the p107 9mer for 34cdc2 Assayed by Inhibition of Histone H1 Kinase Activity

The binding affinity of the 9mer peptide for p34cdc2 was approximated by relating the percent inhibition of histone H1 kinase activity to peptide concentration. Reactions were performed as described in Example 4. The percent inhibition was quantitated using an Ambis Radioanalytic Imaging System. The percent inhibition was plotted versus the peptide concentration. In calculating a binding constant, it was assumed that the concen-

11

tration of substrate and peptide were in vast excess of the p34^{cdc2} protein, which was assumed to be limiting in the reaction.

As depicted in Figure 2, the k_d was calculated from the point of 50% inhibition, yielding a $k_d \cong 10$ μM. The binding affinity of the peptide for p34^{cdc2} may be similar to that of the whole molecule due to the ability of the peptide to efficiently inhibit p34^{cdc2} activity, which is directly related to its ability to compete with activating factors.

Example 8

Affinity of the p107 9mer for p34^{cdc2} Assayed by ELISA

An ELISA based assay was developed to measure the activity of p107 peptides and their derivatives. A 96-well cluster plate coated with the p107 30mer binds to p34^{cdc2} kinase from cell extracts. This binding is easily detectable with anti-p34^{cdc2} antibodies and can be used to quantitate the affinity of the p107 wild type and mutant peptides for the p34^{cdc2} kinase in addition to the kinase inhibition assay described in Example 7. A summary of all modifications to the original p107 peptides and their effect on binding and kinase inhibition is presented in Table 1. The affinity of the p107 9mer peptide for p34^{cdc2} kinase is approximately 10 μM as measured by the ELISA based competition assay, which is in agreement with the binding constant approximated from the kinase inhibition assay as described in Example 7.

Introduction of a non-conservative amino acid substitution within the pentapeptide sequence CAFYI [SEQ ID NO:_____] resulted in substantial decreases in both p34^{cdc2} inhibition and kinase binding except at Cys$_{713}$.

These data demonstrate that p107 protein is capable of associating with p34^{cdc2}. This is consistent with its homology to Rb and the cyclin proteins, and presents a structural basis for the functional similarities shared by these proteins.

Example 9

Inhibition of Human Papillomavirus-E7 Protein

Rb and p107 form specific complexes with the adenovirus E1A oncoprotein and Rb forms a similar complex with the E7 oncoprotein human papillomavirus (HPV). In order to determine whether the p107 30mer peptide domain and Rb may interact with other tumor suppressor proteins or other cellular and viral ligands, p107 30mer peptide binding to cellular and bacterially expressed human papillomavirus (HPV)-E7 protein was examined. p107 30 amino acid peptide-sepharose, prepared as described in Example 2, was incubated with CaSki cell (HPV-16 positive cervical carcinoma) extract and assayed for the binding of E7 protein. Western blot analysis demonstrated that the peptide did bind to HPV16-E7 protein from CaSki cells.

The association of bacterially expressed HPV18-E7 protein with the p107 30mer peptide was analyzed using an ELISA assay. In the experiment depicted in Figure 3A, a 96-well cluster plate was coated with p107 30mer peptide and incubated with increasing concentration of a MBP-E7 fusion protein or the MBP protein as a control. This experiment indicates that the p107 30mer peptide can bind directly to HPV-E7 protein and that binding is not dependent upon virus type. In the experiment depicted in Figure 3B, MBP-E7 fusion protein (100 ng) was incubated with a p107 30mer peptide-coated plate in the presence of increasing concentrations of free p107 9mer peptide. As shown in Figure 3, binding is directly proportional to the increase in the optical density at 405 nm. The results of competition experiments shown in Figure 3B indicate that the affinity of the p107 9mer peptide for the HPV18-E7 protein is approximately 1 nM, which is approximately 10,000-fold greater than for the p34^{cdc2} kinase.

Example 10

ELISA Assay of p107 Competition for E7-Rb Complex Formation

To examine the effect of p107 peptide binding upon the E7-Rb interaction, an ELISA assay was used to monitor E7-Rb complex formation. 96-well cluster plates were coated with either MBP-E7 protein (○) or MBP alone as a control ( ). The plates were incubated with increasing concentrations of baculovirus express d Rb which was detected with monoclonal antibody αRb349 (Pharmingen, San Diego CA) (Figur 4A). In a separate experiment, 96-well cluster plates were coated with MBP-E7 protein and incubated with 100 ng Rb in the pr s-ence of incr asing concentrations of p107 9mer peptide (Figure 4B). Binding is quantitated as an increase in

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optical density at 405 nm. The results of the experiments depicted in Figure 4A clearly demonstrate the ability of bacterially expressed E7-MBP fusion protein to bind to Rb. The results of the experiment shown in Figure 4B demonstrate that addition of increasing concentrations of p107 9mer peptide to the reaction effectively competes for E7 association with Rb. This result suggests that the p107 9mer peptide may be an effective inhibitor of HPV-E7 protein. A corresponding peptide from Rb protein (sequence MCSMYGICK [SEQ ID NO:_____ _____]) also binds to the E7 oncoprotein and competes for the E7-Rb interaction.

Thus, inhibitors of p34^{cdc2} and p33^{cdk2}, as well as inhibitors of human papillomavirus, have been disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT:
        (A) NAME: BRISTOL-MYERS SQUIBB COMPANY
        (B) STREET: 345 PARK AVENUE
        (C) CITY: NEW YORK
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        (H) TELEFAX: 206 727 3601

    (ii) TITLE OF INVENTION: PEPTIDE INHIBITORS OF THE P33CDK2 AND
        P34CDC2 CELL CYCLE REGULATORY KINASES AND HUMAN
        PAPILLOMAVIRUS E7 ONCOPROTEIN

    (iii) NUMBER OF SEQUENCES: 35

    (iv) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Leu Cys Ala Phe Tyr Ile Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid

5   (C) STRANDEDNESS: single
               (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

10

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

        Met Cys Ser Met Tyr Gly Ile Cys Lys
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15
(2) INFORMATION FOR SEQ ID NO:3:

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             (A) LENGTH: 5 amino acids
20   (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

25

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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30   1   5

(2) INFORMATION FOR SEQ ID NO:4:

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             (A) LENGTH: 173 amino acids
35   (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

40

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

        Pro Asp Tyr His Glu Asp Ile His Thr Tyr Leu Arg Glu Met Glu Val
45   1   5   10   15

        Lys Cys Lys Pro Lys Val Gly Tyr Met Lys Lys Gln Pro Asp Ile Thr
                        20   25   30

50   Asn Ser Met Arg Ala Ile Leu Val Asp Trp Leu Val Glu Val Gly Glu

55

15

ISDOCID: <EP   0666270A2 I >

Glu Tyr Lys Leu Gln Asn Glu Thr Leu His Leu Ala Val Asn Tyr Ile
50 55 60

Asp Arg Phe Leu Ser Ser Met Ser Val Leu Arg Gly Lys Leu Gln Leu
65 70 75 80

Val Gly Thr Ala Ala Met Leu Leu Ala Ser Lys Phe Glu Glu Ile Tyr
85 90 95

Pro Pro Glu Val Ala Glu Phe Val Tyr Ile Thr Asp Asp Thr Tyr Thr
100 105 110

Lys Lys Gln Val Leu Arg Met Glu His Leu Val Leu Lys Val Leu Thr
115 120 125

Phe Asp Leu Ala Ala Pro Thr Val Asn Gln Phe Leu Thr Gln Tyr Phe
130 135 140

Leu His Gln Gln Pro Ala Asn Cys Lys Val Glu Ser Leu Ala Met Phe
145 150 155 160

Leu Gly Glu Leu Ser Leu Ile Asp Ala Asp Pro Tyr Leu
165 170

(2) INFORMATION FOR SEQ ID NO:5:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 171 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Glu Tyr Val Lys Asp Ile Tyr Ala Tyr Leu Arg Gln Leu Glu Glu
1 5 10 15

Glu Gln Ala Val Arg Pro Lys Tyr Leu Leu Gly Arg Glu Val Thr Gly
20 25 30

Asn Met Arg Ala Ile Leu Ile Asp Trp Leu Val Gln Val Gln Met Lys
35 40 45

Phe Arg Leu Leu Gln Glu Thr Met Tyr Met Thr Val Ser Ile Ile Asp

```
        50                      55                      60
Arg Phe Met Gln Asn Asn Cys Val Pro Lys Lys Met Leu Gln Leu Val
65                  70                  75                  80

Gly Val Thr Ala Met Phe Leu Ala Ser Lys Tyr Glu Glu Met Tyr Pro
                85                  90                  95

Pro Glu Ile Gly Asp Phe Ala Phe Val Thr Asp Asn Thr Tyr Thr Lys
                100                 105                 110

His Gln Ile Arg Gln Met Glu Met Lys Ile Leu Arg Ala Leu Asn Phe
                115                 120                 125

Gly Leu Gly Arg Pro Leu Pro Leu His Phe Leu Arg Arg Ala Ser Lys
    130                 135                 140

Ile Gly Glu Val Asp Val Glu Gln His Thr Leu Ala Lys Tyr Leu Met
145                 150                 155                 160

Glu Leu Thr Met Leu Asp Tyr Asp Met Val His
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(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 179 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Trp Gln Ser Ser His Tyr Leu Gln Trp Ile Leu Asp Lys Gln Asp Leu
1               5                   10                  15

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            20                  25                  30

Trp Lys Leu Gln Ile Phe Phe Thr Asn Val Ile Gln Ala Leu Gly Glu
            35                  40                  45

His Leu Lys Leu Arg Gln Gln Val Ile Ala Thr Ala Thr Val Tyr Lys
    50                  55                  60

Arg Phe Tyr Ala Arg Tyr Ser Leu Lys Ser Ile Asp Pro Val Leu Met
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17

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5               65                    70                    75                    80

                Ala Pro Thr Cys Val Phe Leu Ala Ser Lys Val Glu Glu Phe Gly Val
                                85                  90                      95

10              Val Ser Asn Thr Arg Leu Ile Ala Ala Ala Thr Ser Val Leu Lys Thr
                            100                 105                     110

                Phe Ser Tyr Ala Phe Pro Lys Glu Phe Pro Tyr Arg Met Asn His Ile
                            115                 120                     125

15              Leu Glu Cys Glu Phe Tyr Leu Leu Glu Leu Met Asp Cys Cys Leu Ile
                        130                 135                 140

                Val Tyr His Pro Tyr Arg Pro Leu Leu Gln Tyr Val Gln Asp Met Gly
                145                     150                 155                 160

20              Gln Glu Asp Met Leu Leu Pro Leu Ala Trp Arg Ile Val Asn Asp Thr
                                165                 170                     175

                Tyr Arg Thr

25
        (2)  INFORMATION FOR SEQ ID NO:7:

             (i)  SEQUENCE CHARACTERISTICS:
                  (A)  LENGTH: 173 amino acids
                  (B)  TYPE: amino acid
30                (C)  STRANDEDNESS: single
                  (D)  TOPOLOGY: linear

             (ii) MOLECULE TYPE: peptide

35

             (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

                Asn Leu Leu Asn Asp Arg Val Leu Arg Ala Met Leu Lys Ala Glu Glu
40              1                   5                   10                      15

                Thr Cys Ala Pro Ser Val Ser Tyr Lys Cys Val Gln Lys Glu Val Leu
                            20                  25                      30

                Pro Ser Met Arg Lys Ile Val Ala Thr Trp Met Leu Glu Val Cys Glu
45                      35                  40                  45

                Glu Gln Lys Lys Cys Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr
                    50                  55                  60

50              Asp Arg Phe Leu Ser Leu Glu Pro Val Lys Lys Ser Arg Leu Gln Leu

55
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5        65                          70                        75                         80

         Leu Gly Ala Thr Cys Met Phe Val Ala Ser Lys Met Lys Glu Thr Ile
                         85                  90                  95

10       Pro Leu Thr Ala Glu Lys Leu Cys Ile Tyr Thr Asp Asn Ser Ile Arg
                         100                 105                 110

         Pro Glu Glu Leu Leu Gln Met Glu Leu Leu Leu Val Asn Lys Leu Lys
                     115                 120                 125

15       Trp Asn Leu Ala Ala Met Thr Ala His Asp Phe Ile Glu His Gly Leu
                 130                 135                 140

         Ser Lys Ile Ala Glu Ala Glu Glu Asn Lys Gln Ile Ile Arg Lys His
         145                 150                 155                 160

20       Ala Gln Thr Phe Val Ala Leu Cys Ala Thr Asp Val Lys
                         165                 170
```

(2) INFORMATION FOR SEQ ID NO:8:

25   (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 174 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

30   (ii) MOLECULE TYPE: peptide

35   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
         Trp Ala Asn Arg Glu Glu Val Trp Lys Ile Met Leu Asn Lys Glu Lys
         1               5               10                  15

         Thr Tyr Leu Arg Asp Gln His Phe Leu Glu Gln His Pro Leu Leu Gln
40                   20                  25                  30

         Pro Lys Met Arg Ala Ile Leu Leu Asp Trp Leu Met Glu Val Cys Glu
                     35                  40                  45

         Val Tyr Lys Leu His Arg Glu Thr Phe Tyr Leu Ala Gln Asp Phe Phe
45           50                  55                  60

         Asp Arg Tyr Met Ala Thr Gln Glu Asn Val Val Lys Thr Leu Leu Gln
         65                  70                  75                  80

50       Leu Ile Gly Ile Ser Ser Leu Phe Ile Ala Ala Lys Leu Glu Glu Ile
```

55

```
                              85                   90                   95

        Tyr Pro Pro Lys Leu His Gln Phe Ala Tyr Val Thr Asp Gly Ala Cys
                    100                 105                 110

        Ser Gly Asp Glu Ile Leu Thr Met Glu Leu Met Ile Met Lys Ala Leu
                    115                 120                 125

        Lys Trp Arg Leu Ser Pro Leu Thr Ile Val Ser Trp Leu Asn Val Tyr
                    130                 135                 140

        Met Gln Val Ala Tyr Leu Asn Asp Leu His Glu Val Leu Leu Pro Gln
        145                 150                 155                 160

        Tyr Pro Gln Gln Ile Phe Ile Gln Ile Ala Glu Leu Leu Asp
                        165                 170
```

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 168 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
        Val Arg Leu Arg Asp Leu Cys Leu Lys Leu Asp Val Ser Asn Glu Leu
        1               5               10                  15

        Arg Arg Lys Ile Trp Thr Cys Phe Glu Phe Thr Leu Val His Cys Pro
                    20                  25                  30

        Asp Leu Met Lys Asp Arg His Leu Asp Gln Leu Leu Leu Cys Ala Phe
                    35                  40                  45

        Tyr Ile Met Ala Lys Val Thr Lys Glu Glu Arg Thr Phe Gln Glu Ile
            50                  55                  60

        Met Lys Ser Tyr Arg Asn Gln Pro Gln Ala Asn Ser His Val Tyr Arg
        65                  70                  75                  80

        Ser Val Leu Leu Lys Ser Ile Pro Arg Glu Val Val Ala Tyr Asn Lys
                        85                  90                  95

        Asn Ile Asn Asp Asp Phe Glu Met Ile Asp Cys Asp Leu Glu Asp Ala
```

<div style="text-align:right">100   105   110</div>

```
Thr Lys Thr Pro Asp Cys Ser Ser Gly Pro Val Lys Glu Glu Arg Ser
        115             120             125

Asp Leu Ile Lys Phe Tyr Asn Thr Ile Tyr Val Gly Arg Val Lys Ser
        130             135             140

Phe Ala Leu Lys Tyr Asp Leu Ala Asn Gln Asp His Met Met Asp Ala
145             150             155             160

Pro Pro Leu Ser Pro Phe Pro His
                165
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 173 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Tyr Leu Arg Leu Asn Thr Leu Cys Glu Arg Leu Leu Ser Glu His Pro
1               5               10              15

Glu Leu Glu His Ile Ile Thr Leu Phe Gln His Thr Leu Gln Asn Glu
                20              25              30

Tyr Glu Leu Met Arg Asp Arg His Leu Asp Gln Ile Met Met Cys Ser
        35              40              45

Met Tyr Gly Ile Cys Lys Val Lys Asn Ile Asp Leu Lys Phe Lys Ile
        50              55              60

Ile Val Thr Ala Tyr Lys Asp Leu Pro His Ala Val Gln Glu Thr Phe
65              70              75              80

Lys Arg Val Leu Ile Lys Glu Glu Glu Tyr Asp Ser Ile Ile Val Phe
                85              90              95

Tyr Asn Ser Val Phe Met Gln Arg Leu Lys Thr Asn Ile Leu Gln Tyr
                100             105             110

Arg Pro Pro Thr Leu Ser Pro Ile Pro His Ile Pro Arg Ser Pro Tyr
```

5

	115	120	125

Lys Phe Pro Ser Ser Pro Leu Arg Ile Pro Gly Gly Asn Ile Tyr Ile
    130   135   140

10

Ser Pro Leu Lys Ser Pro Tyr Lys Ile Ser Glu Gly Leu Pro Thr Pro
145   150   155   160

Thr Lys Met Thr Pro Arg Ile Leu Val Ser Ile Gly Glu
           165   170

15

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 amino acids
        (B) TYPE: amino acid

20
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

25

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Phe Glu Phe Thr Leu Val His Cys Pro Asp Leu Met Lys Asp Leu Met
1   5   10   15

30
Lys Asp Arg His Leu Asp Trp Leu Leu Leu Cys Ala Phe Tyr Ile Met
         20   25   30

Ala Lys

35

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 30 amino acids
        (B) TYPE: amino acid

40
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

45

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Phe Glu Phe Thr Leu Val His Cys Pro Asp Leu Met Lys Asp Arg His
1   5   10   15

50

55

```
      Leu Asp Gln Leu Leu Leu Cys Ala Phe Tyr Ile Met Ala Lys
                   20              25              30
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Asp Leu Met Lys Asp Arg His Leu Asp Gln Leu Leu Leu Cys Ala Phe
1               5                   10              15

Tyr Ile Met Ala Lys
             20
```

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Lys Asp Arg His Leu Asp Gln Leu Leu Leu Cys Ala Phe Tyr Ile Met
1               5                   10              15

Ala Lys
```

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 14 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single

```
                (D)  TOPOLOGY: linear

        (ii)  MOLECULE TYPE: peptide



        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:15:

        Leu Asp Gln Leu Leu Leu Cys Ala Phe Tyr Ile Met Ala Lys
        1               5                   10

  (2)  INFORMATION FOR SEQ ID NO:16:

        (i)  SEQUENCE CHARACTERISTICS:
             (A)  LENGTH: 7 amino acids
             (B)  TYPE: amino acid
             (C)  STRANDEDNESS: single
             (D)  TOPOLOGY: linear

        (ii)  MOLECULE TYPE: peptide



        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:16:

        Ala Phe Tyr Ile Met Ala Lys
        1               5

  (2)  INFORMATION FOR SEQ ID NO:17:

        (i)  SEQUENCE CHARACTERISTICS:
             (A)  LENGTH: 5 amino acids
             (B)  TYPE: amino acid
             (C)  STRANDEDNESS: single
             (D)  TOPOLOGY: linear

        (ii)  MOLECULE TYPE: peptide



        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:17:

        Tyr Ile Met Ala Lys
        1               5

  (2)  INFORMATION FOR SEQ ID NO:18:

        (i)  SEQUENCE CHARACTERISTICS:
             (A)  LENGTH: 7 amino acids
             (B)  TYPE: amino acid
```

```
                    (C)  STRANDEDNESS: single
                    (D)  TOPOLOGY: linear

         (ii)  MOLECULE TYPE: peptide


         (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:18:

         Leu Cys Ala Phe Tyr Ile Met
         1               5

(2)  INFORMATION FOR SEQ ID NO:19:

         (i)  SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 6 amino acids
                    (B)  TYPE: amino acid
                    (C)  STRANDEDNESS: single
                    (D)  TOPOLOGY: linear

         (ii)  MOLECULE TYPE: peptide


         (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:19:

         Leu Cys Ala Phe Tyr Ile
         1               5

(2)  INFORMATION FOR SEQ ID NO:20:

         (i)  SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 5 amino acids
                    (B)  TYPE: amino acid
                    (C)  STRANDEDNESS: single
                    (D)  TOPOLOGY: linear

         (ii)  MOLECULE TYPE: peptide


         (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:20:

         Leu Cys Ala Phe Tyr
         1               5

(2)  INFORMATION FOR SEQ ID NO:21:

         (i)  SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 4 amino acids
```

25

```
        (B)  TYPE: amino acid
        (C)  STRANDEDNESS: single
        (D)  TOPOLOGY: linear

   (ii)  MOLECULE TYPE: peptide




   (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:21:

    Cys Ala Phe Tyr
    1

(2)  INFORMATION FOR SEQ ID NO:22:

        (i)  SEQUENCE CHARACTERISTICS:
            (A)  LENGTH: 9 amino acids
            (B)  TYPE: amino acid
            (C)  STRANDEDNESS: single
            (D)  TOPOLOGY: linear

        (ii)  MOLECULE TYPE: peptide




        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:22:

    Leu Phe Ala Phe Tyr Ile Met Ala Lys
    1                   5

(2)  INFORMATION FOR SEQ ID NO:23:

        (i)  SEQUENCE CHARACTERISTICS:
            (A)  LENGTH: 9 amino acids
            (B)  TYPE: amino acid
            (C)  STRANDEDNESS: single
            (D)  TOPOLOGY: linear

        (ii)  MOLECULE TYPE: peptide




        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:23:

    Leu Cys Glu Phe Tyr Ile Met Ala Lys
    1                   5

(2)  INFORMATION FOR SEQ ID NO:24:

        (i)  SEQUENCE CHARACTERISTICS:
```

          (A) LENGTH: 9 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

     Leu Cys Ala Ala Tyr Ile Met Ala Lys
     1   5

(2) INFORMATION FOR SEQ ID NO:25:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 9 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

     Leu Cys Ala Lys Tyr Ile Met Ala Lys
     1   5

(2) INFORMATION FOR SEQ ID NO:26:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 9 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

     Leu Cys Ala Tyr Tyr Ile Met Ala Lys
     1   5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 9 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Leu Cys Ala Phe Ala Ile Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 9 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Leu Cys Ala Phe Phe Ile Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 9 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x Si)EQUENCE DESCRIPTION: SEQ ID NO:29:

Leu Cys Ala Phe Tyr Lys Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:30:

28

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

    Leu Cys Ala Phe Tyr Ile Met Gln Lys
    1   5

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

    Pro Lys Lys Lys Arg Lys Val Cys Ala Phe Tyr Ile
    1   5   10

(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

    Arg Arg Cys Ala Phe Tyr Ile
    1   5

(2) INFORMATION FOR SEQ ID NO:33:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

    Arg Lys Arg Cys Ala Phe Tyr Ile
    1   5

(2) INFORMATION FOR SEQ ID NO:34:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

    Ser Pro Lys Lys Gly Cys Ala Phe Tyr Ile
    1   5   10

(2) INFORMATION FOR SEQ ID NO:35:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

    Cys Ala Phe Tyr Ile Gly Ser Pro Lys Lys
    1   5   10

Claims

1. An inhibitor of p34^{cdc2} cell cycle regulatory kinase activity, or an inhibitor of the kinase activity of a homolog thereof, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin.

2. The inhibitor according to claim 1, wherein the homolog is p33^{cdk2}.

3. An inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin.

4. The inhibitor according to any one of claims 1 to 3, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a retinoblastoma protein.

5. The inhibitor according to any one of claims 1 to 3, wherein the inhibitor is derived from a p34^{cdc2} binding domain of p107.

6. The inhibitor according to any one of claims 1 to 3, wherein the inhibitor is derived from a p34^{cdc2} binding domain of cyclin E.

7. The inhibitor according to claim 4, wherein the inhibitor is derived from the alpha-helix II domain of a retinoblastoma protein.

8. The inhibitor according to claim 5, wherein the inhibitor is derived from the alpha-helix II domain of p107.

9. The inhibitor according to claim 6, wherein the inhibitor is derived from the alpha-helix II domain of cyclin E.

10. The inhibitor according to claim 7, wherein the inhibitor comprises a peptide including the amino acid sequence MCSMYGICK [SEQ ID NO: 2], or substitutions of the sequence which retain inhibitory activity.

11. The inhibitor according to claim 7, wherein the inhibitor comprises a peptide including the amino acid sequence LCAFYIMAK [SEQ ID NO: 1], or substitutions of the sequence which retain inhibitory activity.

12. The inhibitor according to claim 8, wherein the inhibitor comprises a peptide including the amino acid sequence CAFYI [SEQ ID NO: 3], or substitutions of the sequence which retain inhibitory activity.

13. The inhibitor according to any one of claims 1 to 12, wherein the inhibitor comprises a peptide mimetic.

14. A complex comprising:
    (a) p34^{cdc2} or a homolog thereof; and
    (b) an inhibitor of p34^{cdc2} cell cycle regulatory kinase activity, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin, and wherein the complex substantially lacks kinase activity.

15. The complex according to claim 14, wherein the homolog is p33^{cdk2}.

16. A complex comprising:
    (a) human papillomavirus E7 protein; and
    (b) an inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a p34^{cdc2} binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin.

17. The complex according to any one of claims 14 to 16, wherein the inhibitor is derived from p34^{cdc2} binding domain of a retinoblastoma protein.

18. The complex according to any one of claims 14 to 16, wherein the inhibitor is derived from a p34^{cdc2} binding domain of p107.

19. The complex according to any one of claims 14 to 16, wherein the inhibitor is derived from a p34^{cdc2} binding domain of cyclin E.

20. The complex according to claim 17, wherein the inhibitor is derived from the alpha-helix II domain of a retinoblastoma protein.

21. The complex according to claim 18, wherein the inhibitor is derived from the alpha-helix II domain of p107.

22. The complex according to claim 19, wherein the inhibitor is derived from the alpha-helix domain of cyclin E.

23. The complex according to claim 20, wherein the inhibitor comprises a peptide including the amino acid sequence MCSMYGICK [SEQ ID NO: 2], or substitutions of the sequence which retain inhibitory activity.

24. The complex according to claim 20, wherein the inhibitor comprises a peptide including the amino acid sequence LCAFYIMAK [SEQ ID NO: 1], or substitutions of the sequence which retain inhibitory activity.

25. The complex according to claim 21, wherein the inhibitor comprises a peptide including the amino acid sequence CAFYI [SEQ ID NO: 3], or substitutions of the sequence which retain inhibitory activity.

26. The complex according to any one of claims 14 to 25, wherein the inhibitor comprises a peptide mimetic.

27. A method of inhibiting p34^{cdc2} cell cycle regulatory kinase activity, or the kinase activity of a homolog thereof, comprising:
(a) providing an inhibitor according to any one of claims 1 to 13; and
(b) contacting p34^{cdc2} or the homolog thereof with an inhibiting amount of the inhibitor.

28. A method of inhibiting human papillomavirus E7 activity, comprising:
(a) providing an inhibitor according to claim 3 or any one of claims 4 to 13 when dependent on claim 3; and
(b) contacting human papillomavirus E7 with an inhibiting amount of the inhibitor.

29. An inhibitor according to claim 3 or any one of claims 4 to 13 when dependent on claim 3, for use in inhibiting human papillomavirus E7 activity.

30. A pharmaceutical composition comprising an inhibitor according to any one of claims 1 to 13.

```
CYC  A   175 PDYHEDI-HTYLRENEVKCKPLVGLHKKD-PD-ITNS-MRAILVDWLMEVGEYRLQNETLHLAVN
CYC  B1  167 SEYVKDI-YAYLRQLEEEQ-AVRPKYLLGREV-TGN--MRAILIDWLMQVQMHFLLQETMYMTVS
CYC  C    26 WQSSHYLQWILDKQDLLEERQKDLKFLSEEEY---WKLQIFFTNMICALGEHIHIHRQQVIATAT
CYC  D    21 NILNDRVLFANLKAERTCAPSVSYKCVQKEVL--PS-MRKIVATWMLEVCEEQHCEEEVHLLNH
CYC  E    94 WANREEVDKNLRFITYLRDQHFLEQHPL-LCPK-MRAILIDWLMEVHIHFTFYLAQD
p107     668 VFLRDLQLKLDVSN-ELRRKIWTC-FEFTLV-HCPDLMKDFHIDQLL-LHAHIHARVT--KEER
Rb       661 Y-LFLNILCERLLS-FHISLEEIYLFEQHLLQNEYDLMRDFHIDQIM-MSNIGICKVK-IDID
```

```
CYC  A   DRFLISMSVLHCLLQLVGTAAMLLASKLEELYPPLVAEVY-YIDDTYTKKQVL----ERMER
CYC  B1  DRFMQNNCVLKKNLQLVGVTAMFLASKIEEMYPPLIGDFAF-MLDNTYTKHQIR----QMEM
CYC  C   KRLYASE-SLKSIDPVLMAPTCVFLASKYEEFGVVSNTLIAA-ATSVLKTFSYRPDFFERMNH
CYC  D   DRFLQSLEBVKKSLQLLGATCMFYASKMKETIPLTAELCI--YTDNSIHPEELL-------QMEL
CYC  E   DRYMATQENVVKTLLQLIGISSLFAAKLEELYPPLHDFAY-MIDGACSGDEIL------TME L
p107     QEIMHSYRNCPQANSBVYR-LSVLLCIPREVVAYNKNINDDFEMID-LCLLEDAT----EKTPDC
Rb       BIIVTAYKDLFBRVQETFK-RVLK--EFEEDSIVFYNSVFMCBLKTNILQYRPSILL--SPIE
```

```
CYC  A   LRMLTFDLAAHIVNCR-LTOYTLHQQPANCKVESLAMFLGELSIIDADPYE 248
CYC  B1  LBALNFGLGRPIFLRE-LRRALSKIGEVDVEQHTLAKYLMELTMLDYDMVH 338
CYC  C   ECEFYLLELMDCCIV-YHEIRPLLQYVQDMGQEDMLLPLAWRIVNLTYR 206
CYC  D   VNKLFWNLAAMTAHIF-IEHGLSKIAEAEENKDIIRKHAQTFVALCATDVK 94
CYC  E   MEALKWRL-SPILVSWINVYMQVAYLNDLHEVLLPDYPQQIFIDIAHLIB 368
p107     SGFVREER-EOLIIFYNTIYYSRVKSFALKFDCANDDHMMDAPPLSFFPR 835
Rb       FESPYKFPSSPIRLPG-CNIYITPLKSPYKITEEIPTPTKMTPRILVSIGE 832
```
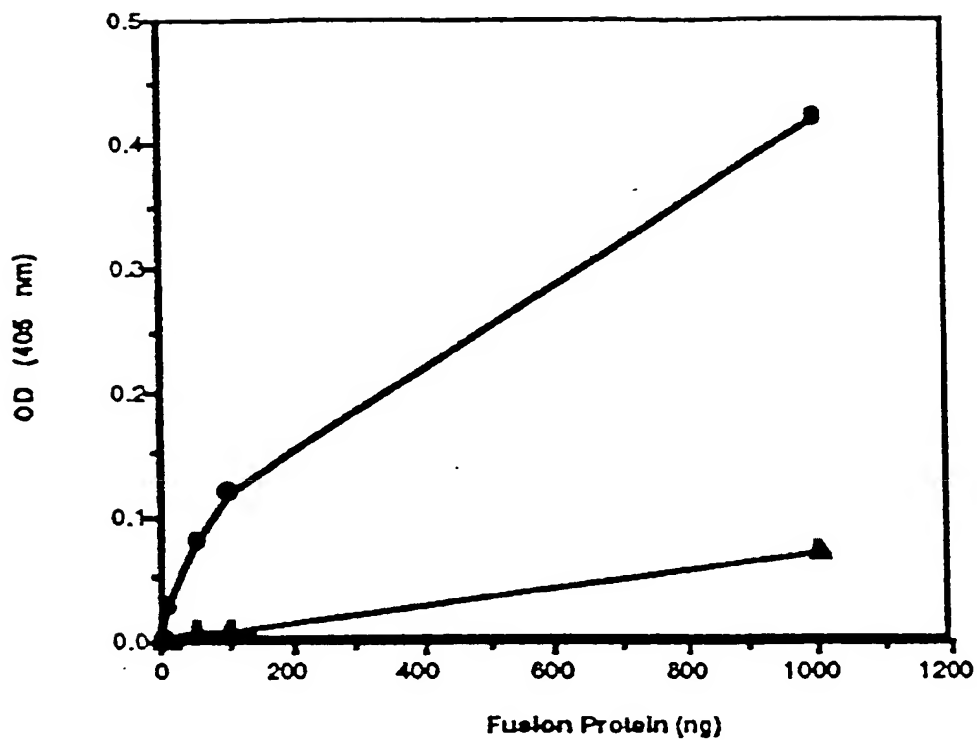
Figure 1

Figure 2

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Figure 3A

Figure 3B

Figure 4A

Figure 4B

(12) **EUROPEAN PATENT APPLICATION**

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(30) Priority: 08.02.1994 US 193977

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(54) **Peptide inhibitors of the p33cdk2 and p34cdc2 cell cycle regulatory kinases and human papillomavirus E7 oncoprotein**

(57) Novel peptide and peptide mimetic ligands which act as inhibitors of p34cdc2 kinase, p33cdk2 kinase and human papillomavirus transforming protein E7 (HPV E7) are disclosed. The inhibitors are derived from the binding domains of a retinoblastoma tumor suppressor protein (Rb), p107 and a cyclin.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 95 30 0736

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WO-A-89 06703 (DRYJA, T.P. ET AL.) 27 July 1989 * claims 5,17,18; fig. 5-2; page 7, lines 13-17 *	1-4,7, 10,30	C07K7/04 C07K14/47 A61K38/02 C12N9/12
X,D	CELL, vol. 66, 1991 pages 1155-1164, EWEN, M.E. ET AL. 'Molecular cloning, chromosomal mapping, and expression of the cDNA for p107 ...' * summary; introduction; p.1159, last paragraph; fig. 5; discussion *	1-5,7,8, 10-12,30	
X	GENES DEV., vol. 7, 1993 pages 111-1125, ZHU, L. ET AL. 'Inhibition of cell proliferation by p107, a relative of the retinoblastoma protein' * figure 7; discussion *	1-3,5,8, 11,12, 14,18, 21,24, 25,27,30	
D,X	NATURE, vol. 346, 1990 pages 760-763, PINES, J. & HUNTER, T. 'Human cyclin A is adenovirus E1A-associated protein p60 ...' * summary *	1-3,6,9, 14,15, 19,27,30	**TECHNICAL FIELDS SEARCHED** (Int.Cl.6) C07K A61K C12N
X	PROC. NATL. ACAD. SCI. USA, vol. 86, 1989 pages 6474-6478, BERNARDS, R. ET AL. 'Structure and expression of the nurine retinoblastoma gene and characterization of its encoded protein' * introduction; fig. 3; discussion * --- -/--	1-4,7, 10, 14-16, 20,23, 27-30	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	24 November 1995	Hermann, R

EPO FORM 1503 03.82 (P04C01)

CATEGORY OF CITED DOCUMENTS

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L : document cited for other reasons

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 95 30 0736

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,A	CELL, vol. 58, 1989 pages 981-990, GIORDANO, A. ET AL. 'A 60kd cdc2-associated polypeptide ...'		
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	24 November 1995	Hermann, R

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D : document cited in the application
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& : member of the same patent family, corresponding document

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